Reproductive cycle of the Ozark zigzag salamander, *Plethodon dorsalis angusticlavius* (Caudata, Plethodontidae), in north central Arkansas

Walter E. MESHAKA, Jr. * & Stanley E. TRAUTH **

* Archbold Biological Station, P. O. Box 2057, Lake Placid, Florida 33852, U.S.A.

** Department of Biological Sciences, *Arkansas State University, State University, Arkansas 72467, U S.A

The reproductive cycle of the Ozark zigzag salamander, *Plethodon dorsalis angusticlavius*, was studied from January to December 1987. Sexual maturity was attained at the end of the second year of life (in early fall) for both sexes. Unlike northern populations of small plethodons, seasonal reproduction was annual for both sexes. Mean clutch size was 5.3 ova, and yolked follicles were present from January to May. The diameters of the vasa deferentia were greatest from January to April. Only adult Ozark zigzag salamanders were found on cedar glades and only during the mating season. Differential use of the cedar glade during courtship was suggestive of a migration, a phenomenon not previously reported for this species or its sibling species.

INTRODUCTION

Plethodon dorsalis angusticlavius is a small woodland salamander averaging 60-98 mm in total adult length (CONANT & COLLINS, 1991) and occurs almost entirely within the Ozark Mountains of the Interior Highlands region in the United States (DOWLING, 1956) The geographic range of the nominotypical subspecies, *P. d. dorsalis*, is within the northeastern United States and disjunct with that of *P. d. angusticlavius* (CONANT & COLLINS, 1991). However, the range of *P. d. dorsalis* comes into contact with that of its sibling species, *P websteri*, of the southeastern United States (HIGHTON, 1979, 1985).

Reproductive characteristics of *P. websteri* reported by SEMLITSCH & WEST (1983) are similar to those of *P. d. angusticlavius* from a location farther north than that of the present study (WILKINSON et al., 1993). Little else has been published regarding the life history of *P. d. angusticlavius* anywhere in its range, and the natural history of *P d. dorsalis* within its range remains unknown. In this study, we present data on the reproductive cycle of *P. d. angusticlavius* in a different habitat located between those of

SEMLITSCH & WEST's (1983) study on *P. websteri* in South Carolina and WILKINSON et al.' (1993) study of *P. d. angusticlavius* in northern Arkansas.

MATERIALS AND METHODS

Salamanders were collected during the day from January to December 1987 at two localities in north central Arkansas. City Rock Bluff (T17N, R11, S31) in Stone County and Calico Rock (T17N, R11W, S28) in Izard County are comprised of cedar glades bordered by oak-hickory forest, and each joins a system of rocky bluffs along the White River which separates the sites by 3 km.

Salamanders were found under large rocks on the cedar glades and 100-150 m away under wet leaf litter along the dripline of the rock formations at the bluffs. All specimens were killed in a dilute chloretone solution (20 %) within 24 hours of capture, fixed in 10 % formalin, and stored in 70 % ethanol. All measurements were taken from specimens preserved at least 30 days. Snout-vent length (SVL) was measured from the tip of the snout to the anterior end of the vent to the nearest 0.1 mm with vernier calipers.

The number and diameters of follicles and the diameter of the anterior region of the right testis and vas deferens were measured with a dissecting microscope and ocular micrometer. Color of testes and the presence or absence of hypertrophied mental glands were noted. Maximum number of eggs a female might lay was determined by counting the largest set of follicles. Females were considered sexually immature if their ovaries contained only white previtellogenic ova and if the females were smaller in body size than the smallest females which contained vitellogenic ova.

The cloacal tissue containing the spermatheca of 45 females was excised and trimmed for either transverse or sagittal sectioning (HUMASON, 1979). Relative amounts of sperm present within the sections of spermathecal sacs were visually estimated as empty or at least half-full. All specimens are deposited in the Arkansas State University Museum of Zoology. Two standard errors ($\pm$ 2 SE) accompany mean measurements.

RESULTS

MALE REPRODUCTION

The testes and vasa deferentia of 54 males (SVL = 39.2 + 0.410 mm) exhibited seasonal variation in size (Table I) Spermatogenic activity was probably greatest during late summer prior to emergence of *P. d. angusticlavius* from summer retreats in October At that time the anterior region of the testes, swollen with sperm, was dark in color and at maximum diameter ($\bar{x}$ = 1.7 $\pm$ 0.063 mm). The anterior region of the testes began to regress in December and was smallest in May ($\bar{x}$ = 0.616 + 0.055 mm).

Table I. – Monthly mean diameters (mm) of the anterior region of the right testes and vasa deferentia in *Plethodon dorsalis angusticlavius*. Means are followed by 2 standard errors. No salamanders were found from June to September.

Month	N	Testes	Vasa deferentia
January	4	1.02 ± 0.14	0.28 ± 0.04
February	10	1.00 ± 0.23	0.33 ± 0.06
March	10	0.92 ± 0.27	0.33 ± 0.06
April	3	0.81 ± 0.11	0.33 ± 0.10
May	13	0.57 ± 0.16	0.16 ± 0.05
October	5	1.70 ± 0.14	0.17 ± 0.05
November	8	1.69 ± 0.15	0.26 ± 0.02
December	1	1.42	0.31

Evacuation of sperm from testes in an antero-posterior direction was accompanied by a concomitant increase in diameters of the vasa deferentia (Table I). By May the vasa deferentia had also reached their smallest diameter ($\bar{x}$ – 0.156 ± 0.016 mm) and by October they had enlarged slightly ($\bar{x}$ = 0 173 ± 0 023 mm). Production of spermatophores occurs when the vasa deferentia are packed with sperm (FRANCIS, 1934). An increase in the diameter of the vasa deferentia occurred from December through April From January through April, coinciding with mating, males were found almost exclusively on the cedar glade habitat. After May mature males could not be found anywhere until October.

FEMALE REPRODUCTION

The ovarian follicles of 60 mature females (SVL = 41.3 ± 0 501 mm) exhibited seasonal variation in size (fig. 1). Ovarian follicles were smallest ($\bar{x}$ = 1.14 ± 0.020 mm) after emergence of females from summer retreats. Follicles steadily increased in size and were largest in early May ($\bar{x}$ = 2.68 ± 0.110 mm), after which time no females could be found until October. The average number of follicles was 5.3 ± 0.125 mm (range. 3-9, N = 267), and clutch size did not significantly increase with SVL ($r = 0.131$, $p > 0.05$).

Histological sections of spermathecae from 45 females revealed a seasonal presence of sperm from January through May (fig. 1), during which time adult females were found almost exclusively on the cedar glade habitat. Spermatophores were detected in histological sections of spermathecae in January and March. Most spermathecae were full of sperm during May and none contained sperm from October to December. No sperm was present in non-reproductive females indicating that insemination generally occurred only in females containing follicles of at least 1.7 ± 0.066 mm (TRAUTH, 1984).

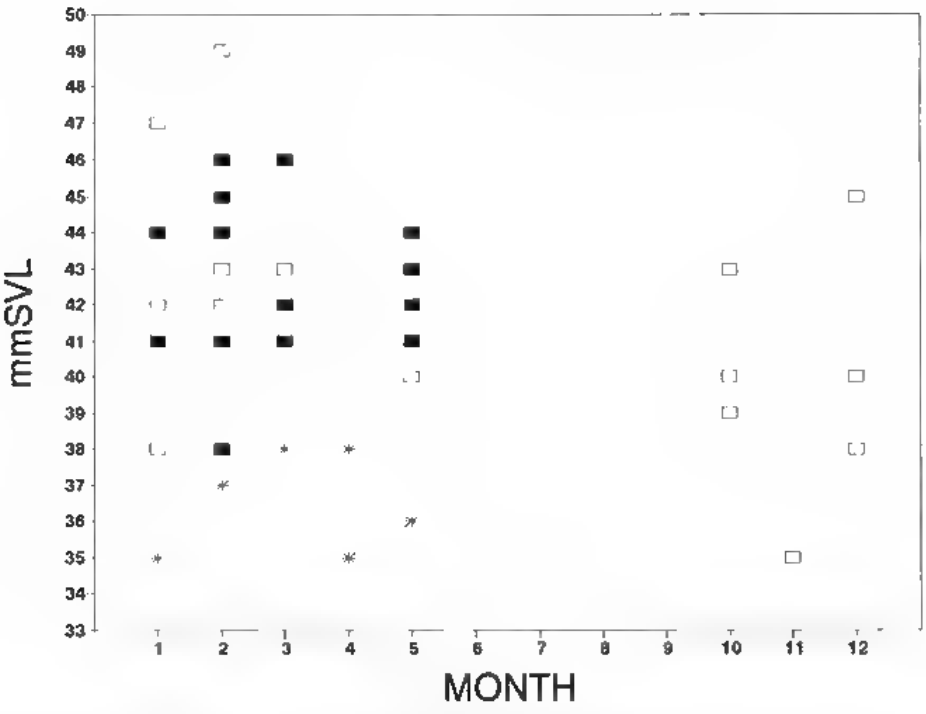

Fig 1. – Seasonal variation in the presence of sperm in the spermatheca of mature female *Plethodon dorsalis angusticlavius* Squares represent mature females Closed squares indicate the presence of sperm. Asterisks represent nonreproductive females.

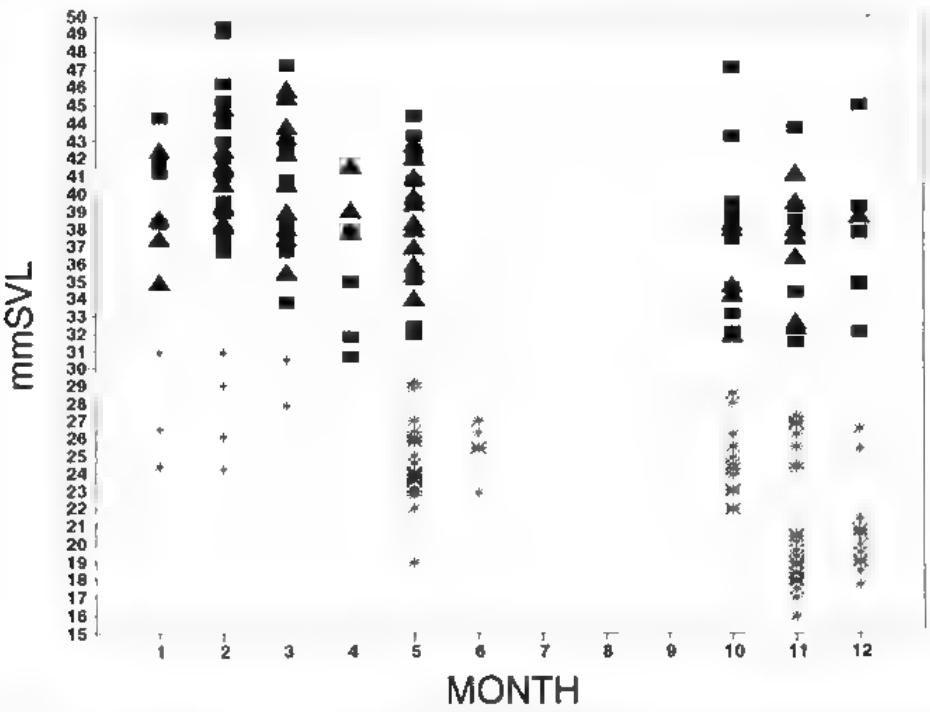

Fig 2 – Size distribution in *Plethodon dorsalis angusticlavius*. Closed triangles represent males. Closed squares represent females. Asterisks represent immatures.

GROWTH AND MATURITY

The smallest *P. d. angusticlavius* had a SVL of 15.9 mm (in November), and it showed no remnants of abdominal yolk. Inaccessibility of hatchlings prior to November precluded an accurate estimation of growing dates; however, a conservative growth rate of approximately 0.76 mm per month was estimated if at least 25 months were necessary to grow 19.0 mm (from 16.0 mm at hatching to a minimum of 35.0 mm at maturity). Salamanders reproduce for the first time beginning January during their third year of life (fig. 2). Average SVL's of mature males and females were not significantly different ($p \geq 0.05$).

SEASONAL ACTIVITY

Immature and mature salamanders were abundant in the wet leaf litter at the base of the bluffs in October. In November and December, mature salamanders began to appear under stones on the cedar glades 100-150 m from the bluffs. From January to April, adults were at the peak of reproductive readiness and presumably were courting During this time interval, adults were found almost exclusively on the cool, wet, cedar glades. Adults were observed in groups of up to four individuals under the many flat stones of this habitat. *Plethodon albagula*, occasionally encountered in the wet leaf litter, was never observed on the cedar glade.

In May, a few adults were found on the cedar glades. By June, adults were absent from the increasingly xeric and hot cedar glades but could be found in the leaf litter of the bluffs. Juveniles were never found on the cedar glades throughout this study. From July through September, salamanders of all sizes could not be found anywhere despite vigorous searching (fig. 2)

DISCUSSION

Growth during the first year of life in *Plethodon dorsalis angusticlavius* was at least 9 mm in SVL and, as in *P. websteri* (SEMLITSCH & WEST, 1983), individuals exhibited a low juvenile growth rate when compared to larger plethodontids (HOUCK, 1977). A long growing season accompanied by small adult size allowed these individuals to reach sexual maturity by the end of their second year. As in *P. websteri* (SEMLITSCH & WEST, 1983) and more northern *P. d. angusticlavius* (WILKINSON et al., 1993), adult size at the onset of sexual maturity was the same for both sexes.

Distinct gonadal cycles in both sexes and the absence of enlarged ovarian follicles in less than 4 % of reproductively mature females was suggestive of a seasonal and annual reproductive cycle. Both reproductive traits are found in *P. websteri* (SEMLITSCH & WEST, 1983) and presumably in more northern *P. d. angusticlavius* (WILKINSON et al., 1993) which have long active seasons. In contrast, northern and western populations of large and small

plethodons, such as *P. glutinosus* (HIGHTON, 1962), *P. cinereus* (SAYLER, 1966), *P richmondi* (ANGLE, 1969), *P. vehiculum* (PEACOCK & NUSSBAUM, 1973), *P. larselli* (HERRINGTON & LARSON, 1987) and *P. ouachitae* (TAYLOR et al , 1990), exhibit a biennial pattern of reproduction. Further, both sexes of the smaller forms mentioned above possess larger minimum body sizes at sexual maturity and mean adult body sizes at northern latitudes where the growing season is shorter.

By December, vasa deferentia were packed with sperm which is associated with production of spermatophores (FRANCIS, 1934). Although December matings were possible, no sperm or spermatophores were detected within the cloacal chambers of females until January. BLANCHARD (1928) stated that the spermatophore cap is expelled soon after mating. Consequently, our data suggest that the mating season began in January and terminated in April.

The courting season of *P. d. angusticlavius* was different than the fall or spring courtship seasons of many northern *Plethodon* populations (HIGHTON, 1962; SAYLER, 1966; ANGLE, 1969; PEACOCK & NUSSBAUM, 1973; HERRINGTON & LARSON, 1987). Winter-spring courtship of *P. d. angusticlavius* was possible probably because of the mild, wet winters with which southern plethodons are associated (SEMLITSCH & WEST, 1983; CAMP, 1988; WILKINSON et al., 1993; this study)

No hatchlings were collected until November in 1987, but the presence of some larger hatchlings in November as well as the emergence of postreproductive females in October indicated that emergence of adults and the smallest hatchlings could occur in October Visits to both collection sites in October 1988 yielded hatchlings and postreproductive females in the leaf litter, which corroborated our suggestion of an October emergence. In wetter years and/or sites, a September emergence is just as feasible.

Comparisons of our results corroborated no latitudinal differences in the reproductive cycle or active season within the small range of this subspecies (WILKINSON et al., 1993) nor with its sibling species of the Southeast (SEMLITSCH & WEST, 1983). However, we did detect a difference in reproductive phenology with respect to an adjoining habitat which was structurally unlike that associated with eastern small plethodons Unlike those of *P. websteri* (SEMLITSCH & WEST, 1983), courting adult *P. d. angusticlavius* of this study had access to cedar glades, a distinct habitat 100-150 m from the rocky bluffs. This habitat was differentially used concurrent to the mating season of *P. d. angusticlavius*. Because we did not mark animals, we are not certain that a breeding migration to the adjoining cedar glade habitat had taken place. However, two lines of evidence suggest that this phenomenon had occurred. First, individuals present on the cedar glade habitat were exclusively adults. Secondly, at the peak of the courting season, very few adults could be found anywhere but the cedar glade habitat.

Although the proximate causes for the purported migration phenomenon are unknown, one observation may yield some insight. During the seasonally cool wet months corresponding with courtship, the microhabitat under the flat rocks of the cedar glade, like the wet leaf litter, was amenable to the presence of salamanders. Two major differences were observed between these two habitats. First, noticeably smaller numbers of predators and numbers of predator species were found under the rocks on the cedar glade than in the bluff system. Perhaps the cedar glade was a safer habitat for courtship activities.

Second, in contrast to the heavily-canopied bluff, the cedar glade received full exposure to the sun. Warmth, held in the heated sandstone, may have created a preferred thermal microhabitat for the courtship activities of this subspecies. Little detail is provided by WILKINSON et al. (1993) regarding habitat characteristics of their site from a county less than 300 km north of our two study sites. Thus, we cannot be certain if differential use of the cedar glade habitat is unique to *P. d. angusticlavius* or just to some populations.

The reproductive cycle and seasonal activity of *P. d. angusticlavius* were almost identical to that of another population of conspecifics and its sibling species, *P. websteri*. Unlike *P. websteri*, adult *P. d. angusticlavius* in our study were found almost exclusively in a different habitat during the courting season. Perhaps the seasonally predator-poor nature of the crevice-like (i.e. surface rock-to-substrate interface) cedar glade habitat and an amenable microhabitat beneath the sandstone may have been responsible for this phenomenon.

RESUMEN

El ciclo reproductivo de la salamandra *Plethodon dorsalis angusticlavius* fue examinado en el periodo desde enero hasta diciembre 1987. Madurez sexual fue alcanzada al final del segundo año de vida (el principio del otoño) en los dos sexos. Contrario a poblaciones nortenas de pequeños plethodons, la reproduction fue anual en los dos sexos. El número medio de la puesta es de 5 3 huevas y huevas vitelogenicas fueron producidos de enero a mayo. Los diametros de las vasa deferentia son mas grandes de enero a abril. Solamente las salamandras adultas fueron encontradas en cedros herbosos y solamente durante la epoca de reproducción. El uso diferencial de cedros herbosos sugiere una migración, un fenómeno no previamente reportado en esta especie o en su especie hermana, *P. websteri*.

ACKNOWLEDGMENTS

This manuscript was extracted from a thesis presented to Arkansas State University for partial fulfillment of MS degree to WEM in 1988. WEM extends his appreciation to other committee members V R MCDANIEL and J K. BEADLES for their support throughout time spent at ASU. Fondest appreciation goes to W. E and R A MESHAKA who have been a constant source of encouragement in the endeavors of the senior author Both authors acknowledge B P BUTTERFIELD, P MCLARTY, and the late R. L COX for their camaraderie and assistance in the field. Ronn ALTIG kindly reviewed an earlier version of this manuscript

LITERATURE CITED

ANGLE, J P , 1969 The reproductive cycle of the northern ravine salamander, *Plethodon richmondi richmondi*, in the valley and ridge province of Pennsylvania and Maryland *J. Wash. Acad. Sci.*, **59**: 192-202.

BLANCHARD, F. N., 1928. – Topics from the life history and habits of the red-backed salamander in southern Michigan. *Amer. Nat.*, **62**: 156-164.

CAMP, C. D., 1988 Aspects of the life history of the southern red-back salamander *Plethodon serratus* Grobman in the southeastern United states. *Amer. Midl. Nat.*, **119**: 93-100.

CONANT, R. & COLLINS, J. T., 1991 *A field guide to reptiles and amphibians of eastern and central North America*. Boston, Houghton Mifflin Company: 1-450.

DOWLING, H. G., 1956 – Geographic relations of Ozarkian amphibians and reptiles. *Southw. Nat.*, **4**: 174-189.

FRANCIS, E. T., 1934 – *The anatomy of the salamander*. Oxford, The Clarendon Press: 1-60.

HERRINGTON, R. E. & LARSON, J. H., Jr., 1987. – Reproductive biology of the Larch Mountain salamander (*Plethodon larselli*). *J. Herpet.*, **21**: 48-56.

HIGHTON, R. T., 1962. – Revision of the north american salamanders of the genus *Plethodon*. *Bull. Florida State Mus.*, **6** 235-367.

----- 1979. – A new cryptic species of salamander of the genus *Plethodon* from the southeastern United States (Amphibia. Plethodontidae). *Brimleyana*, **1**: 31-36

----- 1985. – The width of the contact zone between *Plethodon dorsalis* and *P. websteri* in Jefferson County, Alabama. *J. Herpet*, **19** 544-546

HOUCK, L. D. 1977. – Life history patterns and reproductive biology of neotropical salamanders. *In*: D. H. TAYLOR & S. I. GUTTMAN (eds.), *The reproductive biology of amphibians*, New York, Plenum Press: 43-72

HUMASON, G. L. 1979. – *Animal tissue techniques*. San Francisco, Freeman: 1-478.

PEACOCK, R. L. & NUSSBAUM, R. A., 1973. – Reproductive biology and population structure of the western red-backed salamander, *Plethodon vehiculum* (Cooper). *J. Herpet*, **7** 215-224.

SAYLER, A., 1966 – The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. *Copeia*, **1966**: 183-193.

SEMLITSCH, R. D. & WEST, C., 1983. – Aspects of the life history and ecology of Webster's salamander, *Plethodon websteri*. *Copeia*, **1983**: 339-346.

TAYLOR, C. L., WILKINSON, R. F., Jr. & PETERSON, C. L., 1990 – Reproductive patterns of five plethodontid salamanders from the Oachita Mountains. *Southw. Nat.*, **35**. 468-472.

TRAUTH, S. E., 1984. – Spermathecal anatomy and the onset of mating in the slimy salamander (*Plethodon glutinosus*) in Alabama. *Herpetologica*, **40**: 314-321.

WILKINSON, R. F., PETERSON, C. L, MOLL, D. & HOLDER, T., 1993 Reproductive biology of *Plethodon dorsalis* in northwestern Arkansas *J. Herpet.*, **27**: 85-87.

Corresponding editor: Günter GOLLMANN.